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Chronic toxicity of indoxacarb and its amelioration by rhizome powder of *Hedychium spicatumin* WLH Cockerels

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Abstract

The objective of the present study was to evaluate the chronic toxicity by determining biochemical parameters after prolong exposure with indoxacarb @ 250 ppm and its amelioration by concurrent administration of rhizome powder of *Hydechiumspicatum* (HSRP) @ 2000ppm and 4000ppm in feed for 16 weeks in WLH cockerels. The total protein, globulins, albumins and glucose levels in blood serum were significantly (P<0.05) decreased whereas direct bilirubin, indirect bilirubin, BUN, creatinine, ALT, AST, ALP, GGT were significantly (P<0.05) increased in indoxacarb intoxicated cockerels groups indicating hepatotoxic and nephrotoxic effects. Simultaneous administration of HSRP in both 2000ppm and 4000pp min diet produced amelioration in biochemical profile of indoxacarb intoxicated cockerels. Thus, it is concluded that HSRP was effective in restoration of biochemical parameters and revealed hepatoprotective and nephroprotective effects in 16 weeks feeding trial in cockerels.

Keywords: Biochemical, Indoxacarb, *Hedychium spicatum*, WLH cockerels

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Introduction

In the living organisms flow of biochemical signaling and chemical energy indicates the complexity of life. Now- a- days, analytical biochemistry has become a tool for successful explaining the living processes which helps in understanding of the biological molecules and their interaction with the exogenous materials. The *Hedychiumspicatum*, commonly known as Gandhpalassi or Kapoor Kachari, is found in abundance around the Himalayan region of Uttrakhand, Assam and Arunachal Pradesh. The plant has been used as hepatoprotective agent [1]and produced immunomodulatory [2,3], cytotoxic [4] and antioxidant effects[5,6]. The major active principles of *H.spicatum* include glycosides, alkaloids, flavonoids, phenolics and other phytoconstituents[7] which produce the effects on liver, GIT, CVS, Nervous system[8] and HSRP have also produced ameliorating potential on haematological parameters in chonic indoxacarb intoxicated cockerels[9].

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Indoxacarb, a new insecticide, belongs to oxidiazine class of pesticides and produces its action by inhibiting the sodium channels[10]. It damages the liver and kidney in rats by generating the reactive oxygen species (ROS) [11,12], immunosuppression by reducing the cell count occurring due to bone marrow suppression and atrophy of lymphoid organs [13,14]. In view of medicinal values of the plants, this study was undertaken to explore the ameliorative potential of *Hedychium spicatum* rhizome powder (HSRP) to restore the biochemical alterations following pronged exposure of indoxacarb in cockerels.

Materials and Methods

Plant materials

The rhizome of *Hedychium spicatum* L. was collected from the Medicinal Research Development Center, G. B. Pant University of Agriculture & Technology, Pantnagar. The fresh rhizomes of the plants were collected, chopped into slices and shade dried for 15-20 days. Later the chopped rhizomes were placed in incubator at 37°C for 2-4 h and grounded in electric grinder to prepare fine homogenous rhizome powder of *Hedychium spicatum* (HSRP). The powder was stored in sealed plastic container in dry place at room temperature till further use.To prepare medicated ration for experimental birds was prepared weekly by mixing HSRP in feed homogenously in required concentration and kept in closed containers. Silymarin tablets (Silybon-140) from Microlab^R was purchased from local market and prepare the ration by mixing the powder of 18 silymarin tablet added and mixed thoroughly in 10 kg feed prepare the required quantity of ration (250 ppm).

Experimental animals and chemicals

Forty nine white leghorn cockerels, 6 to 8 weeks old, weighing 300 to 400 g, were procured from Instructional Poultry Farm of the University and maintained under deep litter system following standard management practices.

Indoxacarb (Avaunt,15.8 % EC) used in the study was purchased from local market and technical grade of indoxacarb (98.4%), from Gardha Chemical Pvt. Ltd., Mumbai, India. Reagents used in this study were procured from Himedia and Sigma and diagnostic kits from Autospan and Erba Diagnostics.

Experimental design

Experimental cockerels were randomly divided into seven groups of 7 birds each and then were kept for 15 day for adaptation. All the seven groups of cockerels were given ration and clean water *ad libitum* throughout the 16 week study at the dietary levels of medication as groups I (Normal grower ration, control), II (indoxacarb (250ppm), III (silymarin 250ppm), IV(indoxacarb @250ppm+silymarin @250ppm),V(HSRP@4000ppm), VI (HSRP@ 2000ppm+ indoxacarb@250ppm) and VII (HSRP@ 4000ppm+ indoxacarb@250ppm). The daily dose of indoxacarb was selected as one tenth of LD_{50}(250mg/kg) which was equivalent to dietary level of 250 ppm. The protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC) vide approval number IAEC/VPT/CVASc/193 dated. 27. 02. 2015.

Biochemical analysis

The blood samples from each bird were collected after 8 and 16 weeks interval of the study from the wing vein and serum was separated and stored at -20°C until assay of different biochemical parameters within 24 h. Enzymic activities of serum aspartate amino transferase (AST), alanine amino transferase (ALT) [15], alkaline phosphatase (ALP) [16] and serum analytes such as BUN [17], creatinine [18], plasma protein, albumin (A) and globulin (G) [17] were determined at 8thweek and termination of study after 16th week of study using diagnostic kits (Autospan and ERBA). Tissue protein was estimated by the method of Lowry [19].

Statistical analysis

Statistical analysis was done using two way analysis of variance (ANOVA) to determine the significance difference ($P < 0.05$) in efficacy of HSRP treatment between two means based on individual observations of different parameters[20].

Results

There was significant ($P<0.05$) reduction of 24.8 and 27.8 %in total serum proteins (g/dL), 23.97 and 18.27 % in albumin (mg/dL) and 28.6 and 33% globulin (mg/dL) in indoxacarb treated group II in comparison to control at 8 and 16 weeks interval respectively. These reduction were significantly ($P<0.05$) increased in HSRP treated group in dose dependent manner and at par with the normal.

A significant (P<0.05) reduction by 13 % and 10.74% of direct bilirubin (mg/dL) was observed in indoxacarb intoxicated group II in comparison to control groups I at 8 and 16 weeks interval, respectively, whereas significant (P<0.05) elevation in indirect bilirubin (mg/dL) by 31% and 26% was observed in indoxacarb intoxicated group II in comparison to control groups I at 8 and 16 weeks interval, respectively (Table 1).

Serum glucose (mg/dL) was decreased significantly (P<0.05 by 8.61 and 10.76 % in indoxacarb treated group II in comparison to control groups I at 8 and 16 weeks interval, respectively. After treatment with HSRP at low and high doses the glucose levels were increased by 2 and 6%, respectively.

The serum creatinine and blood urea nitrogen were elevated significantly (P<0.05) by 13 and 15% and 24 and 20%, respectively, in indoxacarb treated group II in comparison to control groups I at 8 and 16 weeks interval.

The enzymatic activities of serum ALT and AST were significantly (P<0.05) augmented in indoxacarb treated group II by 49 and 50%, respectively, in comparison to control group I at both 8 and 16 weeks intervals (Table 2). Treatment in groups VI and VII with indoxacarb+HSRP at low and high doses and silymarin in group IV showed the significant(P<0.05) reduction of ALT activities upto 15 and 20% in comparison to group II which were close to control.

As shown in Table 2, the significant (P<0.05) elevation in ALP activity by 162 % and 167% in indoxacarb treated group II was observed in comparison to control at 8 and 16 weeks intervals. Simultaneous treatment with HSRP in high and low doses led to significant (P<0.05) reduction upto30-35% and 35-37% in VI and VII at 8 and 16 weeks intervals in comparison to group II and was parallel to the silymarin treated group IV.

There was a significant (P<0.05) elevation of GGT exceeding 129 and 131% in indoxacarb treated group II in comparison to control group I at 8 and 16 weeks intervals (Table 2). HSRP alone did not affect the GGT activity in group V, however, in groups VI and VII simultaneous administration of indoxacarb+HSRP significantly (P<0.05) restored the GGT activities upto 15% and 20% at 8 and 16 weeks interval in comparison to group II.

Discussion

The findings indicating reductions in serum total proteins, globulin and albumin in present study is corroborated with the finding of indoxacarb intoxication in rats[21]. The liver plays an important role in synthesis and secretion of wide range of proteins into the blood stream including blood clotting factors and transporter proteins such as lipoproteins and albumin[22]. The indoxacarb induced hepatic damage resulted due to oxidative stress in mice [12].The elevation of total serum proteins, albumin and globulin in group VI and VII treated with HSRP was similar to silymarin treated group IV due to hepatoprotective efficacy that helped to normalize the total protein, albumin and globulin levels in indoxacarb treated cockerels [23, 1].

The elevation in serum level of direct bilirubin typically results from obstruction either within the liver (intra-hepatic) or outside the liver. that Indoxacarb induced hepatic damage by enhancing the release of free radicals has also been reported earlier which also caused the haemolysis[13] leading to elevation in level of serum direct bilirubin in mice[12]. The decline in serum direct bilirubin by 4 % and 8% due to treatment with HSRP in this study might have been due to hepatoprotective efficacy of HSRP[23,1]. An increase in total and indirect bilirubin is indicative of liver dysfunction as an elevated level of indirect bilirubin occurs after hepatocellular dysfunction [24]. Haemolysis may be attributed for elevation of total serum bilirubin. Indoxacarb produced liver damage by producing oxidative stress in rat[12]and haemolysis by oxidative denaturation of haemoglobin[14]. The decrease in total bilirubin and indirect bilirubin following treatment with HSRP might be due to its hepatoprotective and antioxidant efficacy[23,1].

The indoxacarb induced hepatic and renal damage has also been reported earlier in mice[12] and dogs[14]. The hepatic function is directly associated with the regulation of glucose metabolism and its storage in liver as glycogen and thus affects its reabsorption and excretion. The HSRP induced ameliorative property in hypoglycaemia in indoxacarb induced toxicity condition have occurred due to hepatoprotective and antioxidant property of the plant[23,1].

An increase in the serum levels of urea and creatinine is an indication of probable kidney damage[25]. In addition, increased hepatic urea production from amino acid metabolism could also be responsible for an increase in blood urea nitrogen. Earlier reports also indicated indoxacarb induced damage of liver and kidney [12,14]. An increase in creatinine level suggested the muscle damage and subsequent degradation of muscle

Table 1. Effect on biochemicalparameters following administration of HSRP in feed for 16 weeks in indoxacarb treated WLH cockerels (Mean±SE, n=7).

Groups	Treatment	Total Protein (g/dL)		Albumen (mg/dL)		Globulin (mg/dL)		Direct bilirubin (mg/dL)		Indirect bilirubin (mg/dL)		Glucose(mg/dL)	
		8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks
I	Control	5.24± 0.32^{a}	5.38± 0.25^{a}	1.71± 0.03^{b}	1.86± 0.025^{a}	3.53± 0.39^{a}	3.51± 0.16^{a}	0.29± 0.0056^{b}	0.295± 0.0058^{b}	1.29± 0.041^{a}	1.35± 0.054^{b}	292.69± 1.64^{a}	293.58± 6.65^{a}
II	Indoxacarb (250ppm)	4.10± 0.25^{b}	3.88± 0.26^{b}	1.58± 0.041^{c}	1.52± 0.044^{c}	2.52± 0.26^{b}	2.35± 0.25^{b}	0.32± 0.00871^{a}	0.3267± 0.0053^{a}	1.69± 0.12^{ab}	1.71± 0.040^{a}	267.48± 3.08^{c}	261.99± 5.79^{c}
III	Silymarin (250ppm)	5.41± 0.27^{a}	5.53± 0.24^{a}	1.87± 0.008^{a}	1.91± 0.037^{a}	3.53± 0.32^{a}	3.62± 0.21^{a}	0.29± 0.0032^{b}	0.2958± 0.0030^{b}	1.28± 0.063^{b}	1.29± 0.018^{b}	293.33± 5.57^{a}	294.39± 2.73^{a}
IV	Indoxacarb (250ppm)+ (Silymarin 250ppm)	5.13± 0.14^{a}	5.15± 0.14^{a}	1.70± 0.053^{b}	1.74± 0.045^{b}	3.43± 0.21^{ab}	3.41± 0.19^{a}	0.29± 0.0016^{ab}	0.2925± 0.0025^{b}	1.34± 0.070^{b}	1.35± 0.073^{b}	283.23± 2.45^{ab}	280.20± 6.10^{ab}
V	***H. spicatum* (4000ppm)**	5.34± 0.24^{a}	5.40± 0.23^{a}	1.82± 0.038^{a}	1.89± 0.027^{a}	3.51± 0.30^{a}	3.51± 0.24^{a}	0.29± 0.0046^{b}	0.29± 0.0025^{b}	1.27± 0.059^{b}	1.29± 0.024^{b}	292.87± 4.39^{a}	293.19± 4.63^{a}
VI	**Indoxacarb (250ppm)+ *H. spicatum* (2000ppm)**	5.07± 0.32^{a}	5.08± 0.24^{a}	1.67± 0.035^{b}	1.70± 0.028^{b}	3.39± 0.30^{ab}	3.38± 0.29^{a}	0.31± 0.002^{ab}	0.31± 0.00036^{b}	1.42± 0.045^{b}	1.43± 0.034^{b}	275.09± 8.21^{bc}	274.69± 3.27^{bc}
VII	**Indoxacarb (250ppm)+ *H. spicatum* (4000ppm)**	5.12± 0.26^{a}	5.15± 0.15^{a}	1.74± 0.030^{ab}	1.84± 0.023^{a}	3.38± 0.23^{ab}	3.30± 0.16^{a}	0.2958± 0.0023^{a}	0.2965± 0.0018^{b}	1.38± 0.07^{b}	1.40± 0.094^{b}	284.51± 4.30^{ab}	

Mean values in column bearing different alphabetic superscript a, b and c differ significantly ($p<0.05$) when compared vertically between the groups.

Table 2. Effect on biochemical parameters following administration of HSRP in feed for 16 weeks in indoxacarb treated WLH cockerels (Mean±SE, n=7).

Groups	Treatment	Creatinine (mg/dL)		Serum Urea (mg/dL)		ALT(IU/L)		AST(IU/L)		ALP(IU/L)		GGT(IU/L)	
		8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks	8Wks	16Wks
I	**Control**	1.43± 0.024^{b}	1.44± 0.016^{b}	0.74± 0.012^{b}	0.77± 0.026^{b}	24.89± 0.83^{b}	25.54± 0.96^{b}	78.14± 5.61^{c}	81.24± 3.77^{c}	9.34± 0.58^{b}	7.96± 1.07^{b}	27.38± 1.93^{b}	25.08± 2.12^{b}
II	**Indoxacarb (250 ppm)**	1.62± 0.028^{a}	1.66± 0.055^{a}	0.92± 0.015^{a}	0.93± 0.018^{a}	37.10± 2.59^{a}	38.38± 1.79^{a}	110.82± 2.01^{a}	115.38± 0.64^{a}	15.18± 1.15^{a}	13.31± 1.82^{a}	35.41± 2.34^{a}	33.00± 3.76^{a}
III	**Silymarin (250 ppm)**	1.40± 0.016^{bc}	1.41± 0.035^{bc}	0.73± 0.021^{b}	0.74± 0.014^{b}	24.70± 3.32^{b}	25.59± 1.64^{b}	79.59± 4.23^{c}	81.59± 3.99^{c}	9.40± 0.95^{b}	7.74± 0.82^{b}	27.65± 1.54^{b}	25.80± 2.15^{ab}
IV	**Indoxacarb (250 ppm)+ (Silymarin 250 ppm)**	1.48± 0.026^{ab}	1.51± 0.024^{b}	0.75± 0.023^{b}	0.77± 0.012^{b}	28.67± 2.09^{b}	29.76± 2.52^{b}	90.59± 2.60^{b}	91.05± 2.67^{b}	9.93± 0.81^{b}	8.22± 0.63^{b}	28.11± 1.75^{b}	25.82± 1.88^{ab}
V	***H. spicatum* (4000 ppm)**	1.40± 0.044^{bc}	1.42± 0.025^{bc}	0.73± 0.028^{b}	0.74± 0.018^{b}	24.49± 0.96^{b}	25.82± 1.03^{b}	78.61± 3.39^{c}	80.57± 3.46^{c}	9.29± 0.95^{b}	7.82± 0.71^{b}	27.48± 1.50^{b}	25.07± 1.39^{b}
VI	**Indoxacarb (250 ppm)+ *H. spicatum* (2000 ppm)**	1.55± 0.032^{a}	1.57± 0.027^{a}	0.78± 0.027^{b}	0.80± 0.013^{b}	31.63± 2.72^{ab}	32.87± 3.19a^{b}	93.31± 2.58^{b}	94.38± 2.22^{ab}	10.88± 0.80^{b}	8.67± 0.81^{b}	30.12± 1.65^{ab}	28.50± 1.21^{ab}
VII	**Indoxacarb (250 ppm)+ *H. spicatum* (4000 ppm)**	1.50± 0.029^{ab}	1.52± 0.016^{b}	0.75± 0.022^{b}	0.76± 0.028^{b}	29.89± 2.37^{ab}	30.82± 3.06^{b}	91.18± 2.36^{b}	92.08± 3.06^{b}	9.99± 0.54^{b}	8.42± 0.43^{b}	28.75± 1.90^{b}	26.38± 1.77^{ab}

Mean values in column bearing different alphabetic superscript a, b and cdiffer significantly ($p<0.05$) when compared vertically

phosphocreatinine and failure of renal filtration due to degenerative changes in renal tubules and reduced GFR leading to reduced renal clearance of creatinine leading to persistence of elevated concentration of these substances in blood [26].The reduction in creatinine level in HSRP+ indoxacarb treated group by 4-5% and 7-8% in comparison to group II was parallel to group IV treated with silymarin which indicated hepatoprotective and nephroprotective effect of HSRP.

Estimation of alanine aminotransferase(ALT) or serum glutamic pyruvic transaminase (SGPT) activity is the most frequently relied biomarker of hepatotoxicity. It is predominantly found in hepatocytes, cytoplasm along with other tissues and plays an important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate i.e.,alanine + α ketoglutarate $\xrightarrow{ALT}$ pyruvate + glutamate[27].

Elevation in level of this enzyme in blood occurs due to leakage after liver damage. The estimation of this enzyme is a more specific test for diagnosing hepatic damage, since it is primarily found in the liver [28, 29]. Elevation of ALT and AST in indoxacarb treated cockerels in present study is in agreement with the earlier findings in which subacute toxicities of indoxacarb in buffalo calves [30, 31] and toxicities of indoxacarb in broiler chickens[32].

The AST is one of the most frequently used indicators of hepatocellular necrosis. Aspartate aminotransferase (AST, formerly known as serum glutamate oxaloacetic transaminase-SGOT) catalyzes the transfer of the amino acids of aspartate to the keto group of ketoglutaric acid. AST is present in a wide variety of tissues of the heart, skeletal muscle, kidney, brain and liver. Thus, estimation of AST in blood is not an appropriate marker for diagnosis of liver damages[33,34]. Amelioration in ALT and AST activity by HSRP might be due to the presence of polyphenols and other phytoconstituents present in rhizomes of *Hedychium spicatum* which conferred hepatoprotective efficacy to plant[5,1] in CCl_4 induced liver damage in rat[35]. The findings on elevation of ALP by indoxacarb treatment in group II in present study is in agreement with the earlier findings in indoxacarb intoxicated beagle dogs[13], buffalo calves[30,31] and broilers[32].

Alkaline phosphatase releases inorganic phosphate from various organic orthophosphates and is present in nearly all tissues like liver, bone, kidney, intestine and placenta. In liver alkaline phosphatase is found in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes[36]. Average value of alkaline phosphatase varies with age and is relatively high at early ages and at puberty and low at middle age and higher again during old age. Males usually have high values as compared to females[37]. The significant reduction of ALP following treatment with HSRP in this study might have resulted on account of its hepatoprotective effect due to the presence of polyphenols, alkaloids and other phyto-constituents present in rhizomes of *Hedychium spicatum*[5,1].

γ-glutamyl transpeptidase (GGT) is a membrane bound glycoprotein which catalyses the transfer of γ-glutamyl group to other peptides and amino acids. It is present in large amounts in the kidneys, pancreas, liver, intestine and prostate glands[38]. Indoxacarb induced elevation in GGT might have occurred due to hepatic damage which was ameliorated by HSRP due to its antioxidant and hepatoprotective efficacy by the presence of polyphenols, alkaloids and other phyto-constituents present in rhizomes of *Hedychium spicatum*[1,5].

The protective effect of HSRP might be attributed to presence of polyphenolic compound in its rhizomes. Polyphenols act as antioxidant and scavenges reactive oxygen species produced by oxidative damage, thereby diminishing the oxidative radicals induced cytotoxic effect in liver, kidney and other organs. It is concluded from this study that oral administration of indoxacarb @ 250ppm in diet induced chronic toxicity which was characterized by hepatotoxic and nephrotoxic effects; and simultaneous administration of *Hedychium spicatum* rhizome powder (HSRP) @ 2000 and 4000ppm in diet was effective in restoration of hepatic and renal function following a 16 weeks feeding trial in cockerels.

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Conflict of Interest

None Declared

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